RHODORA, Vol. 99, No. 899, pp. 229–240, 1997

INTERSPECIFIC RELATIONSHIPS AND GENETIC DIVERGENCE OF THE DISJUNCT GENUS *LIQUIDAMBAR* (HAMAMELIDACEAE) INFERRED FROM DNA SEQUENCES OF PLASTID GENE *MAT*K

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ABSTRACT. Sequence data of the chloroplast *mat*K gene generated a phylogeny of *Liquidambar* containing two robust clades. One clade consisted of the Chinese species *L. acalycina* and *L. formosana,* while the other was composed of *L. orientalis* from Turkey and the North American *L. styraciflua.* The data support a close relationship between the western Asian and North American species, but not the division of *Liquidambar* into section *Cathayambar* (*L. formosana*) and section *Euliquidambar* (*L. acalycina, L. orientalis* and *L. styraciflua*). Sequence divergence of the *mat*K gene ranged from 0.1 to 1.0% among *Liquidambar* species and the estimated divergence times of the disjunct species in the genus were 45–90 mya, which agrees with the fossil record.

Key Words: phylogeny, *Liquidambar, mat*K, divergence

Liquidambar L. is the only genus in the Hamamelidaceae that has a disjunct distribution with species occurring in western Asia, eastern Asia, and North America. Four species are currently recognized in the genus. *Liquidambar formosana* Hance is widespread in eastern Asia (Chang 1979; Li 1977). *Liquidambar acalycina* Chang is found in at least nine provinces in mainland China (Chang 1979). *Liquidambar orientalis* Mill. occurs in Turkey and some nearby islands such as Rhodes (Rechinger 1943) and Cyprus (Holmboe 1914). *Liquidambar styraciflua* L. is widely distributed in eastern and southeastern North America and southward at high elevations in the mountains of Mexico and Central America to Honduras (Bogle 1986).

In *Liquidambar, L. acalycina* and *L. formosana* differ from *L. orientalis* and *L. styraciflua* in having 3-lobed instead of 5 (–7)-lobed leaves. The leaf lobes of *L. orientalis* are further subdivid-

ed, but they can intergrade with those of *L. styraciflua* (Bogle 1986). The presence of "setae" (Harms 1930), the carpel-like organs in pistillate flowers of *L. formosana,* has been used to distinguish *L. formosana* as section *Cathayambar* Harms from the other *Liquidambar* species as section *Euliquidambar* Harms (Harms 1930; Chang 1979).

Disjunct distribution of closely related plant species has long attracted the attention of both plant systematists and biogeographers (see review in Boufford and Spongberg 1983; Crawford and Lee 1992; Lee and Crawford 1991; Lee et al. 1996; Tiffney 1985a, b; Wen et al. 1996; Wen and Zimmer 1996). For *Liquidambar,* Hoey and Parks (1991, 1994) studied genetic divergence of the four species using allozymes and found that *L. orientalis* was more closely related to *L. styraciflua* than to either of the two eastern Asian species. Their study provided support for the existence of Atlantic land bridges between eastern North America and western Europe in the upper Cretaceous and Tertiary periods.

Crawford et al. (1992) pointed out that DNA sequence data may provide a more precise estimate of divergence than secondary chemicals or allozymes. Recent studies support that suggestion, and have shown that both nuclear and chloroplast DNA sequence data are informative in resolving phylogenetic relationships of disjunct taxa, even though resolution is variable for different taxa at different levels (Kim and Jansen 1994; Suh et al. 1993; Xiang et al. 1994). Undoubtedly, a high resolution of phylogeny provides a foundation for understanding phytogeography of disjuncts (Wen and Zimmer 1996).

The objective of this study is to use DNA sequences of the chloroplast *mat*K gene to investigate genetic divergence of the species of *Liquidambar* at the nucleotide level and to evaluate the phylogenetic and biogeographic relationships among the four *Liquidambar* species.

MATERIALS AND METHODS

Fresh leaves were collected from small trees of *Liquidambar styraciflua, L. acalycina,* and *L. formosana* cultivated in the greenhouse of the University of New Hampshire. Leaf buds of *L. orientalis* were provided by Tracy Omar at the University of Washington Arboretum, Seattle. Leaves of *Mytilaria laosensis*

Table 1. Locations and base compositions of amplification and sequencing primers used in this study. * this primer was synthesized with equal parts of "C" and "T" at base position 6.

Primer	5′ sequence 3′	Designed by
Forward		
*mat*KF1	ACT GTA TCG CAC TAT GTA TCA	Tao Sang
*mat*KF2	GTT CAC TAA TTG TGA AAC GT	Tao Sang
*mat*KF4	ACC CCA CCC CAT CCA TCT	Jianhua Li
*mat*KF5	TGG AGY CCT TCT TGA GCG A*	Jianhua Li
*mat*KF6	TCA GTG GTA CGG AAT CAA ATG C	Jianhua Li
Reverse		
*mat*KR1	GAA CTA GTC GGA TGG AGT AG	Tao Sang
*mat*KR2	TTC ATG ATT GGC CAG ATC A	Tao Sang
*mat*KR2-2	ACG GGG CCA TAA GAA AGT CG	Jianhua Li
*mat*KR3	GAT CCG CTG TGA TAA TGA GA	Tao Sang

Lec. were provided by Zhong-chun Luo at the Forest Bureau of Xinning, Hunan, China.

Total genomic DNAs were extracted from fresh leaves or buds following the protocol of Doyle and Doyle (1987). Polymerase Chain Reaction™ (PCR) was conducted in 0.2 ml thin-walled microcentrifuge tubes. Each 50 μl reaction included 5 μl of 10× *Taq* extender buffer (Stratagene, CA), 4 μl of 2.5 μm dNTP, 4 units (0.8 μl) of *Taq* extender (Stratagene, CA) and *Taq* polymerase (Promega, WI), 1 μl 20 μm primers, 2–3 μl genomic DNA solution (50–100 ng DNA), and an appropriate amount of UV-treated distilled water. The PCR thermocycler program followed Johnson and Soltis (1995) and the primers were *mat*KF1 and *mat*KR1. The PCR products were loaded on 0.8% LMP (Low Melting Point) agarose gel along with lambda *Hind*III DNA size markers and run for 2–3 hours at 40 volts in 0.5× TBE buffer. The band identified by comparison to the markers was then excised from the gel, liquefied at 65°C, and treated with agarase for 30 minutes at 37°C. This gel-purified PCR product was used directly as a sequencing template.

Sequencing reactions were carried out using the Cycle Sequencing Kit and following the manufacturer's protocols (Applied Biosystems, CA). The primers for sequencing were *mat*KF1, *mat*KF2, *mat*KF4, *mat*KF5, *mat*KF6, *mat*KR1, *mat*KR2, *mat*KR2-2 and *mat*KR3 (Table 1). The approximate locations and exact base compositions of the *mat*K primers are shown in Figure

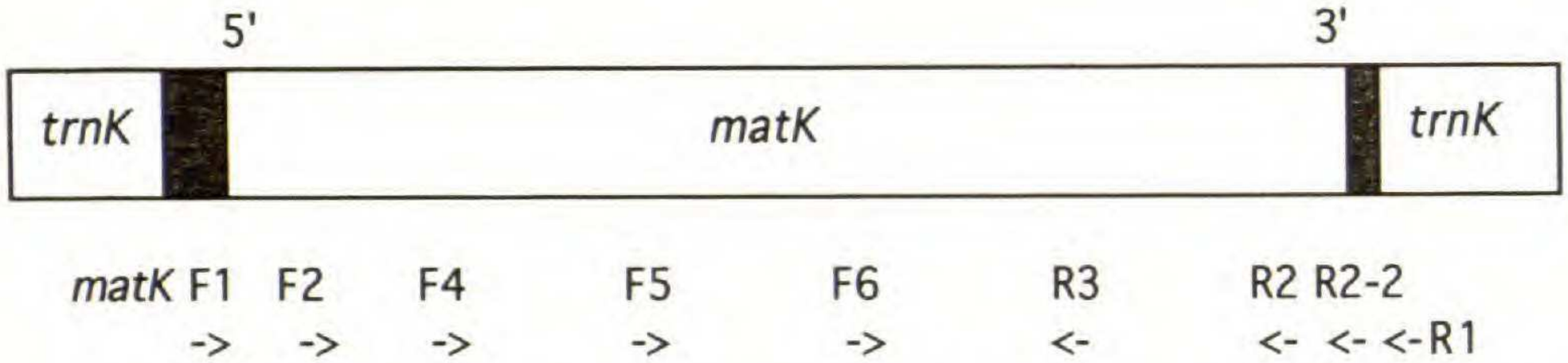

Figure 1. Approximate relative locations of *mat*K primers (base compositions are listed in Table 1, shaded areas are introns).

1 and Table 1. The cycle sequencing products then were separated on 6% polyacrylamide gel using an Automated Sequencer 373A (Applied Biosystems, CA) in the Sequencing Facility Center at the University of New Hampshire. The sequence chromatograms were analyzed using the SEQED program (Applied Biosystems, CA). Also, in order to assure correct basecalling, we overlapped sequences generated from adjacent primers of either the same or opposite directions. The complete *mat*K sequences of *Saxifraga integrifolia* (GenBank accession number L20131) and *Sullivantia sullivantii* (GenBank accession number L20130) were used to determine the limits of *mat*K sequences in *Liquidambar*.

The sequences were readily aligned using the MEGALIGN program of DNA* software packages (DNA* Inc., WI) and by sight. The aligned sequences were imported into the PAUP (Phylogenetic Analysis Using Parsimony) computer program (Swofford 1993) to search for the shortest trees, using the exhaustive search option. Our analysis of intergeneric relationships of the Hamamelidaceae using sequences of internal transcribed spacers (ITS) of nrDNA has shown that *Mytilaria laosensis* is the sister taxon to *Liquidambar* (Li et al., unpubl.). Thus, *M. laosensis* was used as the outgroup in this analysis. All characters and their states were treated equally. The pairwise distances were exported from PAUP and were used for the analysis of divergence. To test the level of clade support, we conducted 500 replicates of bootstrapping (Felsenstein 1985) and decay analysis (Bremer 1988; Donoghue et al. 1992) using the PAUP program. The aligned sequence matrices are available from the first author. The *mat*K sequences of the four species of *Liquidambar* have been submitted to the GenBank and their accession numbers are AF015649 through AF015652.

Studies have not been done previously to estimate substitution rates in the *mat*K gene. Therefore, we estimated a hypothetical

Table 2. *Liquidambar matK* gene sequence divergence (%).

Species	1	2	3	4
L. acalycina	—	0.1	0.5	0.9
L. formosana		—	0.7	1.0
L. orientalis			—	0.5
L. styraciflua				—

rate by using our unpublished data on the divergence between two closely related genera of the Hamamelidaceae—*Dicoryphe* Du Petit-Thours (endemic to Madagascar) and *Trichocladus* Pers. (endemic to eastern and southern Africa; Endress 1989)—and an estimated time of 100 mya, after which Africa and Madagascar were geologically stabilized and direct migration between them was probably not possible (Harland et al. 1990; Raven and Axelrod 1974; Schuster 1976). The resulting estimated substitution rate is 5.5×10^{-11} base per site per year for the *mat*K gene (assuming a constant substitution rate).

RESULTS

Sequence length and divergence. Sequences of the *mat*K gene in *Liquidambar* species were consistently 1512 bases long. Sequence divergences ranged from 0.1 to 1.0% (Table 2). In the aligned sequences there were 90 variable sites, seven of which were found to be phylogenetically informative.

Phylogenetic relationships. A single shortest phylogenetic tree of 92 steps was generated and the consistency index was 1.0 (Figure 2). The tree consisted of two clades, one of which included the east Asian species *Liquidambar acalycina* and *L. formosana,* while the other clade contained *L. orientalis* of western Asia and *L. styraciflua* of North America. Bootstrap percentages and decay indices were 99%/5 and 86%/2 for the two clades, respectively.

Time of divergence. Based on the substitution rate of *mat*K, 5.5×10^{-11} base per site per year (see above), the divergence times for *Liquidambar* species were 9 mya for *L. acalycina* and *L. formosana,* 45 mya for *L. acalycina* and *L. orientalis,* 81 mya for *L. acalycina* and *L. styraciflua,* 90 mya for *L. formosana* and

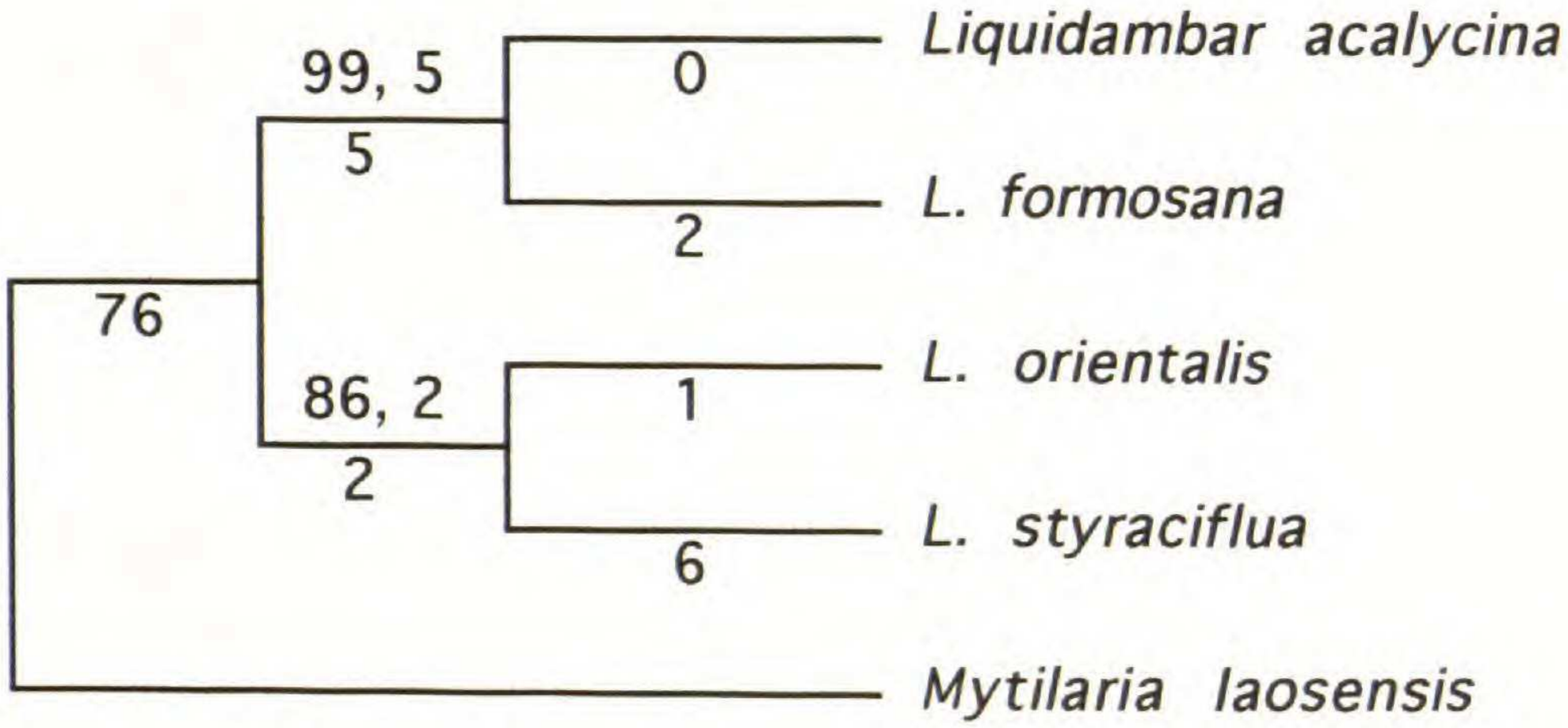

Figure 2. A single shortest phylogenetic tree of 92 steps of *Liquidambar*. Numbers above branches are bootstrap percentages and decay indices, and below branches are branch lengths. Consistency index is 1.0.

L. styraciflua, 63 mya for *L. formosana* and *L. orientalis*, and 45 mya for *L. orientalis* and *L. styraciflua*.

DISCUSSION

How to estimate genetic differences among taxa has long been one of the critical questions about disjuncts in general. Unfortunately, it is very difficult to use morphological, phenological, or even cytological characters to quantitatively assess genetic difference among the disjunct taxa (Lee et al. 1996; Oginuma and Tobe 1991). In contrast, molecular data, especially DNA sequences, have evident advantages over morphological characters in this respect (Crawford et al. 1992). Active research has been carried out in the last several years, using different sources of molecular data to quantify genetic divergence for a variety of disjunct taxa (Hoey and Parks 1991, 1994; Lee et al. 1996; Parks and Wendel 1990; Sang et al. 1994, 1995; Wen and Zimmer 1996; Xiang et al. 1994).

Recent discoveries of fossil flowers attributable to the subtribe Loropetalinae (Hamamelidoideae) have extended the fossil record of the Hamamelidaceae back to the Campanian (~70–75 mya) of the Late Cretaceous (Magallon-Puebla et al. 1996), while flowers of hamamelidaceous affinity extend back to the Santonian (~80 mya; Endress and Friis 1991) and the Turonian (~90 mya; Crepet et al. 1992).

The fossil record of *Liquidambar* and other altingioid plant remains is abundant in the Tertiary, dating back to at least the Tertiary–Late Cretaceous, and possibly to the mid-Cretaceous. Fossil altingioid fruiting inflorescences of the extinct genus *Steinhauera* Presl. are known from the Late Cretaceous–Tertiary boundary (Maastrichtian/Danian) to the Eocene of Europe (Friis and Crane 1989; Mai 1968) and have been considered close to either *Liquidambar* or *Altingia* (Ferguson 1989; Mai 1968; Tiffney 1986). *Liquidambar* pollen was present in the Paleocene (~55–65 mya) of North America and northeastern China (Li et al. 1995; Muller 1981; Taylor 1990; Tiffney 1986; Wang 1992), and Late Cretaceous to Paleocene of China (Sun 1979). Hedlund (1966) reports, with reservation, the possible occurrence of *Liquidambarpollenites* in the Cenomanian of Oklahoma (~95–100 mya).

By comparison, the estimated divergence times (~45–90 mya) for *Liquidambar* species from *mat*K data, using the hypothetical substitution rate described above, were fairly close to the fossil record, indicating an ancient origin and separation of *Liquidambar* populations in disjunct areas of eastern and western Asia, and North America.

As noted in the phylogenetic tree (Figure 2), the two clades are well supported by bootstrap percentages (99% and 86%, respectively). One clade consists of the two east Asian species, *Liquidambar acalycina* and *L. formosana,* and the other includes *L. orientalis* of Turkey and *L. styraciflua* from North America. Thus, the *mat*K phylogeny does not support the recognition of the two sections proposed by Harms (1930) and Chang (1979) based on the presence or absence of setae, and suggests that the presence of setae is an autapomorphy. This pattern, especially the close relationship of the west Asian species *L. orientalis* and North American *L. styraciflua,* has been suggested by an allozyme study (Hoey and Parks 1991). This relationship is also supported by leaf morphology (Bogle 1986).

The divergence times estimated from allozyme data by Hoey and Parks (1991), when Nei's (1987) formula was used, were 7 and 10 mya for *Liquidambar styraciflua–L. orientalis,* and *L. styraciflua–L. formosana,* respectively. However, when Sarich's (1977) and Thorpe's (1982) formulas were adopted, the times for *L. styraciflua–L. orientalis* were 13 and 16 mya, respectively. The estimated divergence times using allozyme data are rather

recent compared to those from *mat*K data and the fossil record (~45–100 mya). Although calibrating the substitution rates is still not certain for either allozyme or *mat*K data sources, allozyme analysis tends to underestimate divergences, especially when amino acid substitutions not affecting electrophoretic mobility go undetected (Crawford et al. 1992). We believe that a comprehensive study of pairs of disjunct taxa whose times of separation have been known more or less precisely, using both nuclear and chloroplast DNA sequences, will provide an invaluable basis for evaluating substitution rates and divergence times of many other taxa.

The *mat*K gene has been widely used in resolving relationships of angiosperms at generic or higher levels (Johnson and Soltis 1995; Soltis et al. 1996; Steele and Vilgalys 1994), but this study suggests that it may be informative also in studying long separated species within a genus even though the substitution rate is low. Nonetheless, due to the low number of informative sites in the *mat*K gene, it seems to be important and interesting to further pursue the phylogenetic relationships of *Liquidambar* species using a fast evolving region such as the internal transcribed spacers of nuclear ribosomal DNA.

In summary, the *mat*K phylogeny supports the close relationship of west Asian *Liquidambar orientalis* and North American *L. styraciflua,* but does not agree with the division of the genus into two sections. The divergence of disjunct *Liquidambar* species was probably at least as early as 45 mya.

ACKNOWLEDGMENTS. The authors wish to express their gratitude to the following people for their help in collecting leaf materials or providing seeds for this study: Zhong-chun Luo, Forest Bureau, Xinning, Hunan, China; Tracy Omar, University of Washington Arboretum, Seattle, WA; and Margaret Hoey, University of North Carolina, Chapel Hill, NC. Special thanks go to T. Sang, Michigan State University, East Lansing, MI, for providing some *mat*K primers, and to two anonymous reviewers for their constructive comments on the manuscript. This study was partially supported by a grant from the Graduate Student Research Enhancement Fund of the University of New Hampshire to JL, and by the Howard and Dorothy Powers Fund to ALB.

LITERATURE CITED

BOGLE, A. L. 1986. The floral morphology and vascular anatomy of the Hamamelidaceae: subfamily Liquidambaroideae. Ann. Missouri Bot. Gard. 73: 325–347.

BOUFFORD, D. E. AND S. A. SPONGBERG. 1983. Eastern Asian-eastern North American phytogeographical relationships—a history from the time of Linnaeus to the twentieth century. Ann. Missouri Bot. Gard. 70: 423–439.

BREMER, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42: 795–803.

CHANG, H.-T. 1979. Hamamelidaceae, pp. 36–116. *In:* H.-T. Chang, ed., Flora Reipublicae Popularis Sinicae, Vol. 35, no. 2. Science Press, Beijing. (in Chinese)

CRAWFORD, D. J. AND N. S. LEE. 1992. Electrophoretic and RAPD divergence between disjunct species and populations of flowering plants in North America and Asia. Amer. J. Bot. 79 (6, suppl.): 6–7 (Abstract).

———, N. S. LEE, AND T. F. STUESSY. 1992. Plant species disjunctions: perspectives from molecular data. Aliso 13: 395–409.

CREPET, W. L., K. C. NIXON, E. M. FRIIS, AND J. V. FREUDENSTEIN. 1992. Oldest fossil flowers of hamamelidaceous affinity, from the Late Cretaceous of New Jersey. Proc. Natl. Acad. Sci. USA. 89: 8986–8989.

DONOGHUE, M. J., R. G. OLMSTEAD, J. F. SMITH, AND J. D. PALMER. 1992. Phylogenetic relationships of Dipsacales based on *rbc*L sequences. Ann. Missouri Bot. Gard. 79: 333–345.

DOYLE, J. J. AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11–15.

ENDRESS, P. K. 1989. Phylogenetic relationships in the Hamamelidoideae, pp. 227–248. *In:* P. R. Crane and S. Blackmore, eds., Evolution, Systematics, and Fossil History of the Hamamelidae. Vol. 1, Introduction and 'Lower' Hamamelidae. Systematics Association Special Volume, No. 40A. Oxford Univ. Press, New York.

——— AND E. M. FRIIS. 1991. *Archamamelis,* hamamelidalean flowers from the Upper Cretaceous of Sweden. Pl. Syst. Evol. 175: 101–114.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

FERGUSON, D. K. 1989. A survey of the Liquidambaroideae (Hamamelidaceae) with a view to elucidating its fossil record, pp. 249–272. *In:* P. R. Crane and S. Blackmore, eds., Evolution, Systematics, and Fossil History of the Hamamelidae. Vol. 1, Introduction and 'Lower' Hamamelidae. Systematics Association Special Volume, No. 40A. Oxford Univ. Press, New York.

FRIIS, E. M. AND P. R. CRANE. 1989. Reproductive structures of Cretaceous Hamamelidae, pp. 155–174. *In:* P. R. Crane and S. Blackmore, eds., Evolution, Systematics, and Fossil History of the Hamamelidae. Vol. 1, Introduction and 'Lower' Hamamelidae. Systematics Association Special Volume, No. 40A. Oxford Univ. Press, New York.

HARLAND, W. B., R. L. ARMSTRONG, A. V. COX, L. E. CRAIG, A. G. SMITH,

AND D. G. SMITH. 1990. A geologic time scale 1989. Cambridge Univ. Press, New York.

HARMS, H. 1930. Hamamelidaceae, pp. 303–345. *In:* A. Engler and K. Prantl, eds., Die Natürlichen Pflanzenfamilien, 2nd ed. Vol. 18A. W. Engelmann, Leipzig.

HEDLUND, R. W. 1966. Palynology of the Red Branch member of the Woodbine formation (Cenomanian), Bryan County, Oklahoma. Bull. Oklahoma Geol. Surv. 112: 1–69.

HOEY, M. T. AND C. R. PARKS. 1991. Isozyme divergence between eastern Asian, North American, and Turkish species of *Liquidambar* (Hamamelidaceae). Amer. J. Bot. 78: 938–947.

——— AND ———. 1994. Genetic divergence in *Liquidambar styraciflua, L. formosana,* and *L. acalycina* (Hamamelidaceae). Syst. Bot. 19: 308–316.

HOLMBOE, J. 1914. Studies on the vegetation of Cyprus, based upon researches during the spring and summer of 1905. Bergens Mus. Skr. 1(2): 1–344.

JOHNSON, L. A. AND D. E. SOLTIS. 1995. Phylogenetic inference in Saxifragaceae sensu stricto and *Gilia* (Polemoniaceae) using *mat*K sequences. Ann. Missouri Bot. Gard. 82: 149–175.

KIM, K.-J. AND R. K. JANSEN. 1994. Comparisons of phylogenetic hypotheses among different data sets in dwarf dandelions (*Krigia,* Asteraceae): additional information from internal transcribed spacer sequences of nuclear ribosomal DNA. Pl. Syst. Evol. 190: 157–185.

LEE, N. S. AND D. J. CRAWFORD. 1991. Electrophoretic divergence between disjunct taxa in eastern Asia and eastern North America. Amer. J. Bot. 78 (6, suppl.): 142 (Abstract).

———, T. SANG, D. J. CRAWFORD, S. H. YEAU, AND S.-C. KIM. 1996. Molecular divergence between disjunct taxa in eastern Asia and eastern North America. Amer. J. Bot. 83: 1373–1378.

LI, H.-L. 1977. Hamamelidaceae, pp. 1–9. *In:* H.-L. Li, T.-S. Liu, T.-C. Huang, T. Koyama, and C. E. DeVol, eds., Flora of Taiwan, Volume III, Angiospermae. Epoch Publ. Co., Taipei, Taiwan.

LI, X., Z. ZHOU, C. CAI, G. SUN, S. OUYANG, AND L. DENG. 1995. Fossil Floras of China Through the Geological Ages. Guangdong Science and Technology Press, Guangzhou, China.

MAGALLON-PUEBLA, S., P. S. HERENDEEN, AND P. K. ENDRESS. 1996. *Allonia decandra:* floral remains of the tribe Hamamelideae (Hamamelidaceae) from Campanian strata of southeastern USA. Pl. Syst. Evol. 202: 177–198.

MAI, D. H. 1968. Zwei ausgestorbene Gattungen im Tertiär Europas und ihre florengeschichtliche Bedeutung. Paleontographica 123: 184–199, Pls. 38, 39.

MULLER, J. 1981. Fossil pollen records of extant angiosperms. Bot. Rev. 47: 1–142.

NEI, M. 1987. Molecular Evolutionary Genetics. Columbia Univ. Press, New York.

OGINUMA, K. AND H. TOBE. 1991. Karyomorphology and evolution in some Hamamelidaceae and Platanaceae (Hamamelididae; Hamamelidales). Bot. Mag. (Tokyo) 104: 115–135.

PARKS, C. R. AND J. F. WENDEL. 1990. Molecular divergence between Asian and North American species of *Liriodendron* (Magnoliaceae) with implications for interpretation of fossil floras. Amer. J. Bot. 77: 1243–1256.

RAVEN, P. H. AND D. I. AXELROD. 1974. Angiosperm biogeography and past continental movements. Ann. Missouri Bot. Gard. 61: 539–673.

RECHINGER, K. H. 1943. Flora Aegaea. Flora der Inseln und Halbinseln des Aegaeischen Meeres. Akad. Wiss. Wien, Math.-Naturwiss. Kl., Denkschr. 105: 1–924.

SANG, T., D. J. CRAWFORD, S.-C. KIM, AND T. F. STUESSY. 1994. Radiation of the endemic genus *Dendroseris* (Asteraceae) on the Juan Fernandez Islands: evidence from sequences of the ITS regions of nuclear ribosomal DNA. Amer. J. Bot. 81: 1494–1501.

———, ———, T. F. STUESSY, AND M. O. SILVA. 1995. ITS sequences and the phylogeny of the genus *Robinsonia* (Asteraceae). Syst. Bot. 20: 55–64.

SARICH, V. M. 1977. Rates, sample sizes, and the neutrality hypothesis for electrophoresis in evolutionary studies. Nature (London) 265: 24–28.

SCHUSTER, R. M. 1976. Plate tectonics and its bearing on the geographical origin and dispersal of angiosperms, pp. 48–139. *In:* C. B. Beck, ed., Origin and Early Evolution of Angiosperms. Columbia Univ. Press, New York.

SOLTIS, D. E., R. K. KUZOFF, E. CONTI, R. GORNALL, AND K. FERGUSON. 1996. *Mat*K and *rbc*L gene sequence data indicate that *Saxifraga* (Saxifragaceae) is polyphyletic. Amer. J. Bot. 83: 371–382.

STEELE, K. P. AND R. VILGALYS. 1994. Phylogenetic analyses of Polemoniaceae using nucleotide sequences of the plastid gene *mat*K. Syst. Bot. 19: 126–142.

SUH, Y., L. B. THIEN, H. E. REEVE, AND E. A. ZIMMER. 1993. Molecular evolution and phylogenetic implications of internal transcribed spacer sequences of ribosomal DNA in Winteraceae. Amer. J. Bot. 80: 1042–1055.

SUN, X.-J. 1979. Palynofloristical investigation on the Late Cretaceous and Paleocene of China. Acta Phytotax. Sin. 17(3): 8–24.

SWOFFORD, D. L. 1993. PAUP: Phylogenetic Analysis Using Parsimony, version 3.1.1. User's Manual. Laboratory of Molecular Systematics. Smithsonian Institution, Washington, DC.

TAYLOR, D. W. 1990. Paleobiogeographic relationships of angiosperms from the Cretaceous and early Tertiary of the North American Area. Bot. Rev. 56: 279–417.

THORPE, J. P. 1982. The molecular clock hypothesis: biochemical evaluation, genetic differentiation and systematics. Ann. Rev. Ecol. Syst. 13: 139–168.

TIFFNEY, B. H. 1985a. Perspective on the origin of the floristic similarity between eastern Asia and eastern North America. J. Arnold Arb. 66: 73–94.

———. 1985b. The Eocene North Atlantic land bridge: its importance in Tertiary and modern phytogeography of the Northern Hemisphere. J. Arnold Arb. 66: 243–273.

———. 1986. Fruit and seed dispersal and the evolution of the Hamamelidae. Ann. Missouri Bot. Gard. 73: 394–416.

WANG, X.-Z. 1992. Palaeopalynological evidence of phylogeny in Hamamelidaceae. Acta Phytotax. Sin. 30(2): 137–145.

WEN, J. AND E. A. ZIMMER. 1996. Phylogeny and biogeography of *Panax* (the ginseng genus, Araliaceae): Inferences from ITS sequences of nuclear ribosomal DNA. Mol. Phylo. Evol. 6: 167–177.

———, R. K. JANSEN, AND E. A. ZIMMER. 1996. Phylogenetic relationships and DNA sequence divergence of eastern Asian and eastern North American disjunct plants, pp. 37–44. *In:* M. Nei and N. Takahata, eds., Current Topics in Molecular Evolution. Pennsylvania State Univ., State College, PA, and the Graduate Univ. for Advanced Studies, Hayama, Japan.

XIANG, Q.-Y., D. E. SOLTIS, AND P. S. SOLTIS. 1994. Phylogenetic relationships and genetic divergence of disjunct taxa from eastern Asia and North America inferred from molecular data: examples from Cornaceae, Hydrangeaceae, and Saxifragaceae. Amer. J. Bot. 81 (6, suppl.): 138–139 (Abstract).